Author's response to reviews

Title: ATBF1 and NQO1 as candidate targets for allelic loss at chromosome arm 16q in breast cancer: Absence of somatic ATBF1 mutations and no role for the C609T NQO1 polymorphism

Authors:

Anne-Marie Cleton-Jansen (a.m.cleton-jansen@lumc.nl)
Ronald Van Eijk (R.van_Eijk@lumc.nl)
Marcel Lombaerts (Lombaerts.m@hsleiden.nl)
Marjanka K. Schmidt (mk.Schmidt@nki.nl)
Laura J. Van 't Veer (l.vt.veer@nki.nl)
Katja Philippo (K.Philippo@lumc.nl)
Rhyenne M.E. Zimmerman (r.zimmerman@chem.leidenuniv.nl)
Hans Peterse (l.vt.veer@nki.nl)
Vincent T.H.B.M. Smit (V.T.H.B.M.Smit@lumc.nl)
Tom Van Wezel (T.van_Wezel@lumc.nl)
Cees J Cornelisse (C.J.Cornelisse@lumc.nl)

Version: 2 Date: 5 February 2008

Author's response to reviews: see over
Dear Editor,

Thank you and the reviewers for the comments on our manuscript. We think we can deal with all issues brought about. Changes to the text are marked yellow in the manuscript and in this letter.

Reviewer 1 (Dr. Ginestier)
1. “The authors should consider further investigation on the three other candidates”
   
   We have shown that one of the candidates (CGI-38) could not be confirmed by RT-qPCR, and discarded this as a candidate. The other two candidates (DBNDD1 and HSBP1) are less likely candidates than NQO1 and ATBF1, which have been implicated in cancer previously, but we agree with the reviewer that they should be considered for further investigation. To address this we have included the following sentence in the discussion:

   “Three other genes were identified to have a significant lower expression in breast tumors with LOH at chromosome 16q. One of these, CGI-38, a brain specific transcript of unknown function could not be confirmed by RT-qPCR. The other two, Dysbindin domain containing 1 (DBNDD1) and Heat shock binding protein 1 (HSBP1) could be confirmed by RT-qPCR and may be subjected to further study in breast cancer.”

2. “Comparison of mRNA level measured by cDNA microarray and qPCR should be shown for each gene analyzed and correlation measured by a statistical test”

   This has been done and presented in the text:

   “For all five genes that showed a significant decrease in expression when comparing breast tumors LOH at 16q, transcript expression was determined by quantitative reverse transcriptase PCR (qPCR)”

   “Correlation between the expression determined by cDNA microarray and RT-qPCR was determined using the statistical correlation function in Microsoft Excel.”

   “In order to confirm the cDNA microarray data quantitative reverse transcriptase PCR (qPCR) was performed on cDNAs from the same samples that were tested on the array. Fig 2 shows the qPCR and microarray data for NQO1 plotted against each other, showing a good correlation of 88%. Correlation for the other genes was 16% for ATBF1, 80% for HSBP1, 79% for DBNDD1 and surprisingly a negative correlation was found for CGI-38, i.e. -31%.”
3. “It would be very interesting to have a figure as a chromosome 16q map, with genes ordered according to their chromosomal location, presenting gene expression profiling and LOH status.”

Such a figure has been include as figure 1 and referred to in the text:

“Fig 1 shows a map of chromosome 16q and the location of these five genes, with their coordinates as determined on the UCSC Genome Browser ([http://genome.ucsc.edu/](http://genome.ucsc.edu/)).”

4. “Discussion line 3-5, the gene identified must be cited.”

The paper that describes this research was already cited, but we have added its name to the text now: **FBXO31**

5. “Please report the primer sequences for the qPCR analyses.”

We do not find this information important to include in the manuscript and therefore state in the text:

“Primer sequences have been submitted to the RTPrimerDB ([http://medgen.ugent.be/rtprimerdb/](http://medgen.ugent.be/rtprimerdb/)).”

Reviewer 2 request:

“It would be of interest to state how the E-cadherin transcript is expresses in this study, if it is included in the cDNA microarray analysis.”

We have checked E-cadherin expression using both cDNA microarray data and RT-qPCR. Herewith the findings as added to the manuscript:

“We also tested whether CDH1, the gene encoding E-cadherin showed significant difference between tumors with and without LOH at 16q, but this was not significant. Correlation between RT-qPCR and microarray for CDH1 was quite good (88%).”